Annexin V-PE/7-AAD Apoptosis Detection Kit

A213

Vazyme

Instruction for Use

Version 23.1

Vazyme

Contents

01/Product Description

Apoptosis is a normal physiological process in embryonic development and maintenance of body homeostasis. Apoptosis has distinct morphological features, including loss of cell membrane asymmetry and attachment, cytoplasmic and nucleoplasmic condensation, and DNA fragmentation between nucleosomes. Disruption of cell membranes is an early feature of apoptosis. Under normal physiologic conditions, phosphatidylserine (PS) is predominantly located in the inner leaflet of the plasma membrane. Upon initiation of apoptosis, PS loses its asymmetric distribution across the phospholipid bilayer and is translocated to the extracellular membrane leaflet marking cells as targets of phagocytosis. Annexin V is a 35 - 36 kDa Ca^{2+}-dependent phospholipid-binding protein with high affinity to PS, so it can bind to the cell membrane of early apoptotic cells through PS exposed on the outside of the cell. Therefore, Annexin V is recognized as one of the sensitive indicators for detecting early apoptosis of cells. Using phycoerythrin (PE) to label Annexin V. The labeled Annexin V retains a high affinity for PS and can be used as a probe to detect apoptosis by fluorescence microscopy or flow cytometry. Since PS eversion occurs in the early stage of apoptosis, Annexin V-PE staining can identify the occurrence of apoptosis in the early stage of apoptosis. 7-Aminoactinomycin D (7-AAD) is a nucleic acid dye and cannot penetrate the intact cell membrane of normal cells or early apoptotic cells. However, 7-AAD can penetrate membrane-damaged cells such as late apoptotic cells or necrotic cells, and bind to their DNA. It can be used to distinguish early apoptotic cells from late apoptotic cells or necrotic cells. Therefore, by co-staining Annexin V-PE with 7-AAD, cells in different apoptotic stages can be distinguished. On the scattergram of dual-color flow cytometry, Annexin V-PE and 7-AAD double negative are normal cells, Annexin V-PE positive and 7-AAD negative are early apoptotic cells, and Annexin V-PE and 7-AAD double positive cells are late apoptotic cells or necrotic cells.

02/Components

Components	A213-01 (50 rxns)	A213-02 (100 rxns)
Annexin V-PE	250 μl	500 μl
7-AAD Staining Solution	250 μl	500 μl
1 × Binding Buffer	25 ml	2 × 25 ml

03/Storage

Store at 2 ~ 8°C. Protect Annexin V-PE and 7-AAD staining solution from light and do not freeze. Adjust the shipping method according to the destination.

04/Applications

It is applicable for the apoptosis detection of adherent cells and suspension cells.

05/Notes

For research use only. Not for use in diagnostic procedures.

1. The apoptosis is a rapid process. It is recommended that samples should be analyzed within 1 h after staining.
2. The occurrence and stage of apoptosis are determined by detecting changes in the cell membrane. Therefore, before Annexin V-PE and 7-AAD staining, fixatives and penetrants that destroy the integrity of the cell membrane cannot be used to fix or penetrate the membrane.
3. During the operation, the action is gentle to avoid mechanical damage to the cells.
4. For adherent cells, digestion is a critical step. If there are floating cells when the adherent cells induce apoptosis, the floating cells and the adherent cells should be collected and stained together. Care should be taken when handling adherent cells to avoid artificial damage. If the trypsin digestion time is too short, the cells need to be vigorously blown and sucked to fall off, which will easily cause damage to the cell membrane and lead to excessive intake of 7-AAD; if the digestion time is too long, the cell membrane is also easy to be damaged, and even affect the bond between PS and Annexin V-PE on the cell membrane. During digestion, spread the trypsin all over the bottom of the well plate, shake gently to make the trypsin fully contact with the cells, then pour out most of the trypsin, and use the remaining small amount of trypsin to digest for a period of time until the space between cells increases, and the bottom of the plate is The variegated can be terminated. Try to use trypsin without EDTA, EDTA will affect the binding of Annexin V to PS.
5. If the sample is from blood, must be sure to remove platelets from the blood. This is because platelets contain PS, which can bind to Annexin V, thereby interfering with the experimental results. Platelets can be washed away by centrifugation at 1,400 rpm (200 × g) using a buffer containing EDTA.
6. Briefly centrifuge the reagent before opening the cap, and shake the liquid on the inner wall of the cap to the bottom of the tube to avoid spilling the liquid when opening the cap.
7. Annexin V-PE and 7-AAD are photosensitive substances, please avoid light during operation.
8. For your safety and health, please wear a lab coat and disposable gloves.

06/Experiment Process

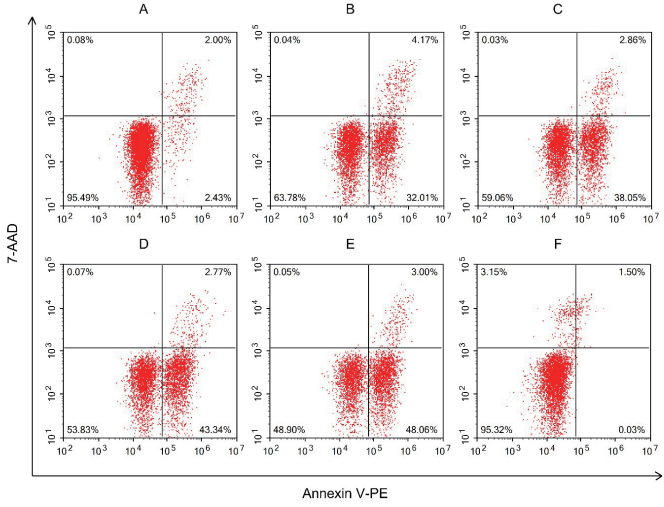
1. Induce apoptosis according to experimental requirements. Untreated cell samples should be included in the test sample as a negative control. In addition, the experimental group needs to be individually stained with Annexin V-PE and 7-AAD to adjust the compensation.
2. Cell collection: collect 1 - 5 × 10^5 cells
   Suspension cells: centrifuge at 1,800 rpm (300 × g) for 5 min at 4°C, discard the culture supernatant;
   Adherent cells: digest the cells with trypsin without EDTA, collect the cells after terminating the digestion, centrifuge at 1,800 rpm (300 × g) for 5 min at 4°C, and discard the supernatant.
3. Cell Washing: wash cells twice with pre-cooled PBS, centrifuge at 1,800 rpm (300 × g) for 5 min at 4°C, discard the supernatant.
4. Cell resuspension: add 100 µl of 1 × Binding Buffer, and gently mix to the single-cell suspension.
5. Cell staining: add 5 µl Annexin V-PE and 5 µl 7-AAD Staining Solution, then mix gently; incubate in the dark at room temperature (20 ~ 25°C) for 10 min; add 400 µl 1 × Binding Buffer and mix gently. The samples were detected by flow cytometry within 1 h after staining.
   ▲ In order to avoid losing cells when washing cells, the front end of the large pipette tip can be added the small pipette tip to aspirate solution.
6. Sample Analysis
   The excitation wavelength of the flow cytometer is 488 nm; the fluorescence of PE is detected in the FL2 channel; the fluorescence of 7-AAD is detected in the FL3 channel and 10,000 events are collected for each sample. Use software such as FlowJo for data analysis. FL2 is the abscissa and FL3 is the ordinate. According to V-PE and 7-AAD fluorescence values, the cut-off between positive and negative cells can be given to set the gate. In a typical experiment, cells can be divided into three subpopulations: living cells are double negative (Annexin V-PE^-/7-AAD^-); early apoptotic cells are Annexin V-PE single positive (Annexin V-PE^+/7-AAD^-); Late apoptotic cells are double positive for Annexin V-PE and 7-AAD (Annexin V-PE^+/7-AAD^+).

07/Examples

Detection of camptothecin-induced apoptosis in Jurkat cells by flow cytometry

Induce Jurkat cells (human T lymphoma cells) to apoptosis with camptothecin (CPT) at the induction concentrations of 0, 4, 8, 12, and 16 µM, respectively. After induction for 4 h, perform staining according to the 06/Experimental Process. The results of flow cytometry are shown in the following figure.

(A) 0 μM. (B) 4 μM. (C) 8 μM. (D) 12 μM. (E) 16 μM. (F) Competition blocking experiment. Induce Jurkat cells with 16 μM CPT for 4 h. Collect and wash cells according to this Instruction. Before staining, add unlabeled Annexin V protein to incubate for 10 min, and then perform Annexin V-PE/7-AAD staining.The dye-free Annexin V bound PS on cells. When restained, Annexin V-PE could not bind PS, indicating the specificity of the staining.



08/FAQ & Troubleshooting

◇ Annexin V-PE staining failure or low positive rate

① The first step is to determine whether the inducer used in the experiment can produce apoptosis. This can be excluded by setting positive drug control with clear apoptosis-inducing effect.

② The most common cause of failure of Annexin V-PE staining for experimental manipulation is improper digestion of adherent cells. The binding of Annexin V to PS requires Ca^{2+}. The Binding Buffer contains 2.5 mM Ca^{2+}. Trypsin digestion with EDTA will affect the staining. It is recommended to use trypsin without EDTA. If trypsin with EDTA is used, the EDTA must be completely removed by washing steps.

③ After washing the cell precipitate with PBS, the residual liquid should be removed as much as possible. The phosphate radicals in the residual PBS will form calcium phosphate precipitates.

④ The cap of the Binding Buffer should be tightly closed to prevent the formation of calcium carbonate precipitation after the entry of CO_2 in the air. This leads to a decrease in free Ca^{2+} and then the experiment fails.

⑤ The density of PS on the cell membrane of some cells is low, and the staining effect is poor. Therefore, it is recommended to change the cell line or use the TUNEL method to detect apoptosis (Vazyme #A112/#A113).

⑥ If it is adherent cells, the floating cells after drug induction should also be collected. These cells are often apoptosis-positive cells, and discarding them will result in lower positive results.

◇ False positive

① In the experiment, it's found that the double positive ratio of Annexin V-PE/7-AAD is too high after staining of the control cells without induced apoptosis. The reason for this result may be the low viability of the cells themselves. It is recommended to use trypan blue staining to calculate the cell viability, and the negative control trypan blue-rejected cells without drug treatment should be greater than 95%. If the cell viability is low, it is recommended to resuscitate the cells. In general, the resuscitated cells should be passaged at least 2 - 3 times before the experiment can be performed.

② Another possibility is that the cells are improperly operated:
a. During the operation, the cells are blown and sucked too vigorously.
b. The digestion time of the adherent cells is too long during the digestion process.
These may lead to the destruction of the cell membrane and false positives.

③ In addition, early apoptosis can occur within a few hours of induction, so the operation should usually be completed within 48 h; if the induction time is too long, nutrients will be exhausted, resulting in poor cell status and high false-positive results.

Nanjing Vazyme Biotech Co.,Ltd.
Tel: +86 25-83772625
Email: info.biotech@vazyme.com
Web: www.vazyme.com
Loc: Red Maple Hi-tech Industry Park, Nanjing, PRC
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